CONTRACTILE PROTEIN FROM CRAYFISH TAIL MUSCLE

K. MARUYAMA

Biological Institute, College of General Education, University of Tokyo,
Komaba, Meguro, Tokyo, Japan

Physicochemical studies on vertebrate striated muscle clearly indicate that the interaction of actomyosin, the muscle contractile protein, with adenosine triphosphate (ATP) may be the fundamental phenomenon on the molecular level in muscular contraction (*cf.* Szent-Györgyi, 1951; Weber and Portzehl, 1954). More and more evidence to support this thesis has been obtained in the field of comparative biochemistry in the animal kingdom (Maruyama and Tonomura, 1957).

In invertebrates, the highly developed muscles of arthropods and molluscs have been investigated biochemically in detail. In arthropods, however, there are very few reports on crustacean contractile protein, whereas the characteristics of insect actomyosin have been well established (Gilmour and Calaby, 1953; Maruyama, 1954, 1957b, 1957c). Edsall and Mehl (1940) investigated flow birefringence properties of lobster myosin in relation to the protein denaturation. Humphrey (1948) prepared myosin from the crab, *Maia,* and briefly described its adenosinetriphosphatase (ATPase) properties. Shrinkage of the glycerine-treated muscle fibers of *Limulus* with ATP was observed by Sarkar (1950). On the physicochemical properties of crustacean tropomyosin, detailed works have been recently published (Tsao, Tan and Peng, 1956; Laki, 1957; Kominz, Saad and Laki, 1957).

The present article is concerned with the results of a comparative biochemical study on the ATP-myosin B system and several associated enzymes in crayfish tail muscle.

MATERIALS AND METHODS

Materials. The crayfish, *Cambarus clarkii,* was used as material. Tail muscles were carefully isolated from exoskeleton and well washed with cold de-ionized water. In a few cases, jaw muscles were also dissected out.

Preparation of contractile protein. The so-called myosin B or natural actomyosin was extracted and purified as established in rabbit skeletal muscle (Szent-Györgyi, 1945). Muscles were homogenized in ten times the volume of cold 0.05 *M* KCl in a Waring Blendor and the water-extractable portion was removed by centrifugating at 3000 G for 5 minutes at 0° C. The residue was washed twice more and finally suspended in five times the volume of the Weber-Edsall solution (0.6 *M* KCl, 0.04 *M* $NaHCO_3$, 0.01 *M* Na_2CO_3). After being placed for 24 hours at 0° C., the suspension turned so viscous that it was diluted with an equal volume of 0.6 *M* KCl. The suspension was centrifuged for 10 minutes to remove the insoluble matter. The viscous supernatant was neutralized to pH 6.5–6.8 with dilute acetic acid and diluted in ten times the volume of cold de-ionized water. The flocculant precipitate was collected by centrifugation, dissolved in 0.6 *M* KCl and diluted in water. This dilution-precipitation procedure was repeated three times and finally the substances insoluble in 0.6 *M* KCl were removed by centrifugation at 8000 G for 30 minutes at 0° C.

Methods of assaying physicochemical properties. The measurements of ultraviolet absorption spectra were carried out with a Shimazu spectrophotometer. Purine and pentose contents were estimated according to Schneider's procedure (Schneider, 1946). Solubility in KCl was tested at pH 7.0 at 0° C., as described in an earlier paper (Maruyama, 1957a). Salting-out analysis was carried out according to Snellman and Tenow (1954), as modified by Tonomura and his associates (1956).

Methods of observing physical changes with ATP. Super-precipitation was observed in a test tube, containing 0.03 *M* tris-(hydroxymethyl)-aminomethane (Tris) buffer, 1 mM ATP and 1.5–2.5 mg. protein at pH 7.0 and 25° C. Viscosity was measured with viscosimeters of the usual Ostwald type at pH 6.4 at 5–10° C. Turbidity was determined in a Hitachi turbidimeter at pH 6.4 and 15° C. For investigation of viscosity and turbidity, the concentration of myosin B was suitably adjusted by dilution with 0.6 *M* KCl. A small amount of concentrated ATP was added to test the ATP effect.

Enzyme tests. The ATPase activity was determined by measuring the increase in inorganic phosphorus (P) after a specified time, usually 5 minutes at 30° C., in a system containing the protein dissolved in 0.6 *M* KCl, 1 mM ATP, 0.033 *M* Tris buffer (pH 7.0), 0.6 *M* KCl, and 10 mM $CaCl_2$ or some other ions, as specified. Total volume of the reaction mixture was 1.5 ml. In order to investigate the effect of pH of incubation, 0.05 *M* histidine was substituted for Tris. The reaction was started by the addition of substrate and stopped by the addition of 0.5 ml. of 20% trichloroacetic acid (TCA). Appropriate blanks were always run simultaneously with the experimentals.

The water-extractable apyrase activity was determined on the 0.05 *M* KCl extract of muscle suspensions. On proving the occurrence of adenylate kinase in crayfish muscle, the 0.05 *M* KCl extract was treated with heat and acid according to Colowick and Kalckar (1943) and incubated in the ATPase-assaying system with and without crayfish myosin B. Adenylate deaminase activity was tested as described before (Maruyama, 1957a).

ATP was purchased as crystalline disodium salt from Sigma and AMP from Schwarz Labs.

Protein was estimated by multiplying nitrogen values, obtained by a micro-Kjeldahl procedure, by the factor 6.

Inorganic phosphorus was measured on a 1.0-ml. aliquot of the TCA supernatant by a micro-modification of the method of Lohmann and Jendrassik (1926), as described by Moriwaki (1956).

RESULTS

Some Physicochemical Properties

Absorption spectra. Ultraviolet absorption spectra of crayfish myosin B dissolved in 0.6 *M* KCl showed the characteristics of protein nature: a maximal absorption was found around 275 mμ and a minimal one was at 255 mμ. Extinction coefficient (ϵ) on basis of gram N per *l*, and 1.0 cm. light path was 9.0 at 275 mμ, which was somewhat higher than that of rabbit myosin B (Tarver and Morales, 1951). The ratio of absorption coefficient at 275 mμ to that at 255 mμ is 1.3.

Purine and pentose contents. The results described above suggest that some minute amounts of purine-containing substances such as nucleotides or nucleic acids were present as contaminants in the preparations. The acid-soluble nucleotide fraction contained 1.5×10^{-5} moles purine per gram myosin B and the nucleic acid fraction contained 1.0×10^{-5} moles. The determinations of pentose showed approximately equimolar amounts and any detectable amounts of desoxyribose were not present. These values are a little higher than those for rabbit myosin B (Buchthal *et al.*, 1951).

Solubility in KCl. Crayfish myosin B was completely soluble in concentrations of KCl higher than 0.4 *M*. The solubility curve is quite in accord with that of rabbit myosin B (Szent-Györgyi, 1945). Myosin B dissolved in 0.6 *M* KCl was slightly yellowish white in color.

TABLE I

Effect of varied concentrations of Ca, Mg and EDTA on the super-precipitation of crayfish myosin B

Concn. *M*	Ca	Mg	EDTA
0	++	++	++
10^{-6}	++	++	++
10^{-5}	++	+++	+
10^{-4}	++	±	±
10^{-3}	+	−	−
10^{-2}	±	−	−

Observed within three minutes after the addition of 1 mM ATP at pH 7.0 and 20° C., in the presence of 0.10 *M* KCl.

+++, ++: intense; +: moderate; ±: weak; −: negative.

Salting-out analysis. Under experimental conditions similar to those of Snellman and Tenow (1954), nearly all the proteins were precipitated between 30–38% of saturated ammonium sulfate in the present preparation.

Physical Changes with ATP

Super-precipitation. Under the optimal conditions, *e.g.*, at 20° C. and pH 7.0 in the presence of 0.10 *M* KCl, a typical super-precipitation was found to take place within one minute after the addition of 1 mM ATP, and to reach the end within five minutes. The super-precipitation was evidently recognized in the presence of KCl in concentrations between 0.06 and 0.16 *M*. The optimal KCl concentrations were 0.10–0.12 *M*, which were quite in accordance with those of rabbit myosin B (Szent-Györgyi, 1945). The effects of Ca, Mg and ethylenediaminetetraacetic acid (EDTA), were tested (Table I). These agents in a high concentration (10 mM) inhibited the super-precipitation. Calcium ions affected little, having no accelerating effect. Magnesium ions greatly speeded up the precipitation in 10^{-5} *M*, but retarded in over 10^{-4} *M*. On the other hand, EDTA inhibited in concentrations higher than 10^{-4} *M*. It should be noted that the inhibitory effects of high concentrations of Mg or Ca and of EDTA were qualitatively different: after several hours, the precipitation took place even in the presence of a high concentration of Mg or Ca, but in the presence of EDTA no precipitation was observed to occur.

Change of viscosity. The relative viscosity of crayfish myosin B dissolved in 0.6 *M* KCl was highly anomalous and ATP caused a marked drop in the viscosity (Fig. 1).

According to Weber and Portzehl (1952), the extent of the viscosity change of actomyosin with ATP (ATP sensitivity) can be expressed as follows:

$$\text{ATP sensitivity} = \frac{Z\eta - Z\eta_{\text{ATP}}}{Z\eta_{\text{ATP}}} \times 100$$

Here $Z\eta = \ln \eta\text{rel}/C$; ηrel = relative viscosity in the absence of ATP; $Z\eta_{\text{ATP}}$ = that in the presence of a sufficient amount of ATP to cause the maximal viscosity change; and C = concentration of protein (grams/liter). The viscosity data derived from Figure 1 are as follows: $Z\eta = 0.40$, $Z\eta_{\text{ATP}} = 0.20$ and the ATP sensitivity = 100%.

The viscosity of myosin B solution drops rapidly with the addition of ATP and gradually rises again when ATP is split by the ATPase action of myosin B. The recovery process followed an S-shaped curve and the recovered viscosity reached about 50% of the drop. Calcium ions, which activate the ATPase action, strongly accelerated the recovery process, while magnesium, an inhibitor of the ATPase action, retarded it. These tendencies are in good accord with those in rabbit or insect myosin B (*cf.* Mommaerts, 1948; Maruyama, 1957b). On the other hand, EDTA, in 10 mM, completely inhibited the viscosity change with ATP.

Change of turbidity. On addition of ATP, the apparent turbidity of myosin B solution decreases because of the decrease of intensity of scattered light (*cf.* Tonomura, 1956). Figure 2 shows the change of turbidity in crayfish myosin B, which

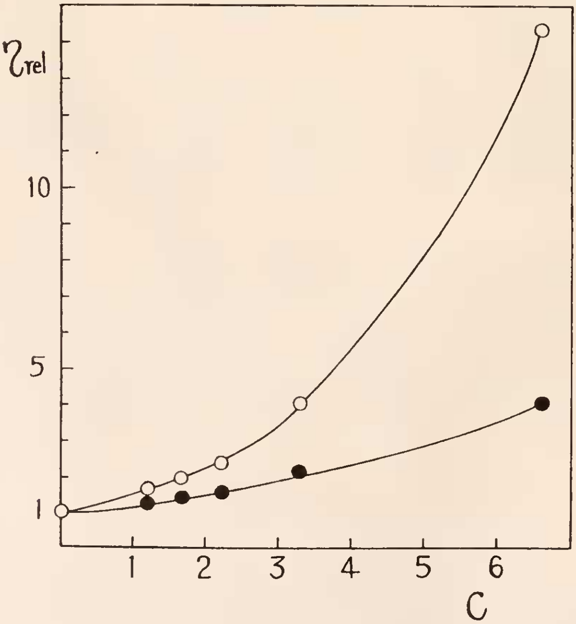

FIGURE 1. Drop of viscosity of crayfish myosin B with ATP; pH 6.4; 6° C.; 0.6 *M* KCl. ○, control; ●, 1 mM ATP added. C, protein concentration, g./*l*.

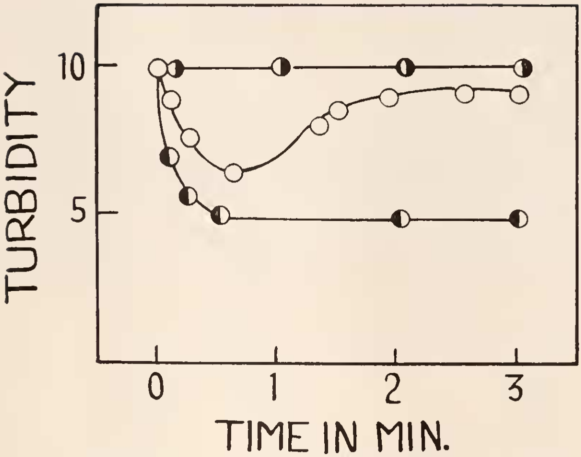

FIGURE 2. Change of turbidity of crayfish myosin B with ATP; pH 6.4; 15° C.; 1 mM ATP; 0.6 *M* KCl. ◑, 10 mM EDTA; ○, 10 mM $CaCl_2$; ◐, 10 mM $MgCl_2$. Turbidity is given as relative level. Time, after the addition of ATP.

is qualitatively similar to that of viscosity described above. The maximal drop of turbidity reached 50% of the original level.

Enzyme Activity

Water-extractable Apyrase

Muscle contains at least two kinds of ATP-splitting enzymes, one myosin itself and the other the water-extractable, magnesium-activated enzyme(s). In crayfish muscle, there was found the latter enzyme(s), too. This fact was already suggested by the classical work of Lohmann (1935) on lobster muscle. The apyrase activity was greatly activated by Mg and completely inhibited by Ca ions. For example, the water-extract, containing 0.3 mg. protein, liberated the following amount of P (μg.) from 2.4 μmole ATP in five minutes at pH 7.0 and 30° C.: 7.0 (none added); 22.0 (5 mM $MgCl_2$); 3.0 (5 mM $CaCl_2$); 2.5 (5 mM $MgCl_2$ plus 5 mM $CaCl_2$). The water-extract of crayfish muscle easily hydrolyzed the two phosphate groups from ATP in the presence of Mg ions.

Adenylate Kinase

In the water-extract of crayfish muscle, adenylate kinase was found to exist. The heat- and acid-treated sample showed no ATPase activity, but in the presence of myosin B, ATPase hydrolyzed the two phosphate groups from ATP. In the presence of 10 mM EDTA, only the terminal phosphate group was set free, even in the combined action of myosin B and the extract. This may be due to the inhibition of adenylate kinase by EDTA, as is well known in rabbit or rat muscle (*cf.* Bowen and Kerwin, 1954).

Adenylate Deaminase

No 5-adenylic acid deaminase activity was detected in crayfish myosin B under the present experimental conditions. This absence of adenylate deaminase activity in crayfish myosin B coincides with the results of Lohmann (1935) on lobster muscle homogenates.

ATPase

Crayfish myosin B possessed a typical calcium-activated ATPase activity, as well as myosin B's from other animal muscles. Preliminary experiments showed that the ATPase action at pH 7.0 was optimal around 30–35° C. and at 37° C. the enzyme was soon inactivated.

Effect of pH. The effect of pH of incubation on the ATPase action of crayfish myosin B is indicated in Figure 3. In the presence of 10 mM $CaCl_2$ in addition to 0.62 *M* KCl and 0.05 *M* histidine, two pH optima were evident: a higher one, at pH 9.0 and a lower one at pH 6.0. In the presence of 10 mM EDTA, a striking activator, a flat, but very high pH optimum between 7–8.5 was found (Fig. 3). The activity level, expressed in terms of Qp (Engelhardt, 1946) is approximately as follows: 1300 at pH 6.0, 600 at pH 7.0 and 2000 at pH 9.0 in the presence of 10 mM $CaCl_2$ and 0.6 *M* KCl at 30° C.; more than 3000 at pH 7–8 in the presence of 10 mM EDTA and 0.6 *M* KCl at 30° C. These values are of a similar magnitude to those reported in actomyosin ATPase from other animals (*cf.* Maruyama and Tonomura, 1957).

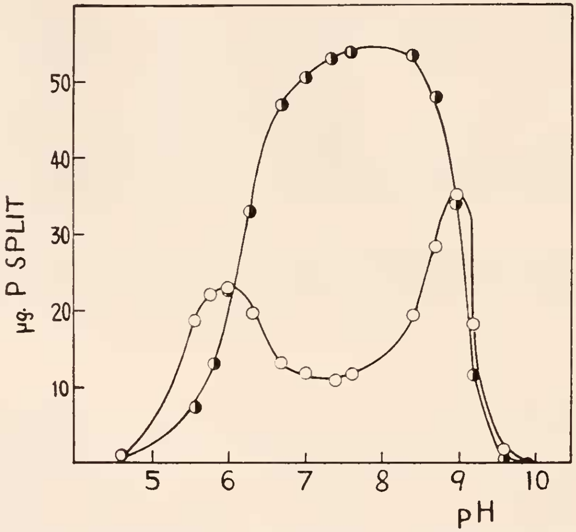

FIGURE 3. Curve of pH-activity of crayfish myosin B ATPase; 0.05 *M* histidine buffer, 30° C.; 5 min. incubation; 0.62 *M* KCl; 2.4 µM ATP; 0.16 mg. protein. ○, 10 mM $CaCl_2$; ◑, 10 mM EDTA.

Effect of enzyme concentration and incubation time. The enzyme activity was found to be linear to enzyme concentration and incubation time, when the hydrolysis of the substrate surpassed no more than half of the added amount. As is seen in Figure 4, it is apparent that in the presence of 10 mM Ca or EDTA, no phosphate was set free after one-half of the labile phosphates corresponding to the terminal phosphate level of ATP were hydrolyzed. In the presence of Mg ions, the activity proceeded at a slight, but constant, rate up to 60 minutes. It is clear that crayfish myosin B exhibits a true ATPase action as well as established in the rabbit.

Effect of Ca, Mg and EDTA. The effects of K, Mg, Ca and EDTA on the ATPase activity of crayfish myosin B were investigated in a systematic way. In dilute KCl concentrations of KCl, *e.g.*, 0.1 *M*, the enzyme action was found to be irregular, possibly owing to the remarkable super-precipitation of the protein. However, it is at least sure that in the presence of 0.1 *M* KCl, $MgCl_2$ at 10^{-5} *M* inhibited the ATPase action, at 10^{-3} *M* it elevated the enzyme activity to the original level and at 10^{-2} *M* it again decreased the activity. In the presence of 0.6 *M* KCl, magnesium depressed the ATPase activity increasingly as the concentration became higher from 10^{-5} to 10^{-2} *M*. On the other hand 0.01–0.1 *M* $CaCl_2$ activated ten times the enzyme action, irrespective of the KCl concentration. Mg competitively inhibited the activating effect of Ca.

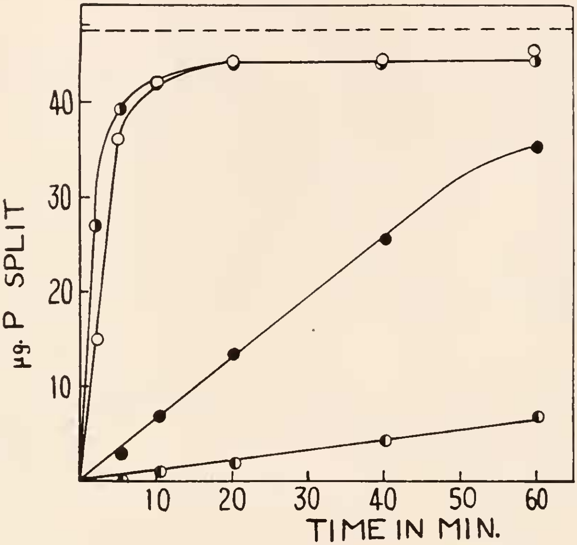

FIGURE 4. Time-activity curve of crayfish myosin B ATPase; pH 7.0; 30° C.; 0.52 *M* KCl; ATP = 48 µg. 1/2 △7P (level of dotted line); 0.5 mg. protein. ●, none added; ◐, 10 mM $MgCl_2$; ○, 10 mM $CaCl_2$; ◑, 10 mM EDTA.

The accelerating effect of EDTA was most remarkable in the presence of 0.6 *M* KCl; 0.6 *M* NaCl could not substitute for KCl. EDTA was inactive in 0.1 mM and gave the strong activation in concentrations higher than 0.5 mM and optimal in 10 mM (see Fig. 5). The effects of Ca, Mg and EDTA on the ATPase activity are seen in Figure 4.

Effect of substrate concentration. The effect of increasing substrate concentration is summarized in Figure 5. The enzyme reaction proceeded according to the Michaelis-Menten theory:

$$\frac{1}{v} = \frac{1}{Vm} + \frac{Km}{Vm}\frac{1}{S}$$

Here v = reaction velocity of first order, Vm = maximal reaction rate, Km = Michaelis constant and S = substrate concentration, Michaelis constant (Km) was approximately 5×10^{-5} *M* (in the presence of 10 mM Ca or 0.1 mM EDTA), 3×10^{-4} *M* (1 mM EDTA) and 1×10^{-3} *M* (10 mM EDTA). Maximal velocity (Vm) also became much greater in the presence of EDTA, as the concentration increased. In the presence of 10 mM Ca ions, Km changed little, although Vm appreciably increased. These facts are in good harmony with those in the rabbit (Tonomura, Matsumiya and Kitagawa, 1957).

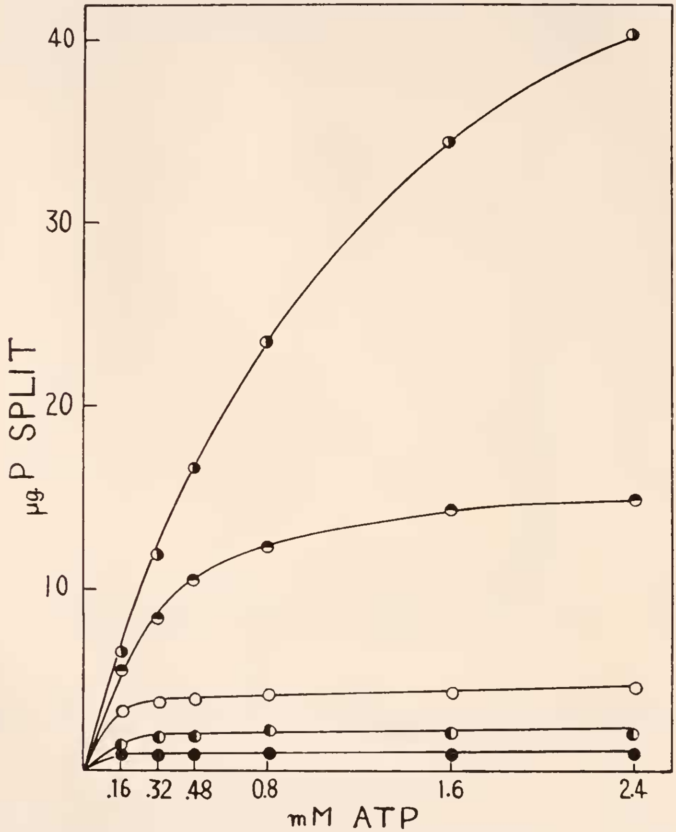

FIGURE 5. Effect of substrate concentration on the ATPase activity of crayfish myosin B; pH 7.0; 30° C.; 3 min. incubation; 0.52 *M* KCl; 0.16 mg. protein. ●, none added; ○, 10 mM $CaCl_2$; ◐, 0.1 mM EDTA; ◓, 1 mM EDTA; ◑, 10 mM EDTA.

Effect of SH reagents. The ATPase activity of crayfish myosin B was very sensitive to heavy metals such as zinc or copper. Especially $ZnSO_4$ in 1 mM completely inactivated the enzyme action. The poisoning action of heavy metals was thought to be mediated through SH groups of myosin B, so that the effect of P-chloromercuribenzoate (PCMB), the powerful SH-blocking agent, was tested; 10^{-4} *M* PCMB nullified the ATPase activity without any pretreatment. After myosin

B was deactivated on a few minutes' pretreatment with 10^{-4} M PCMB, glutathione (GSH) was added and after 5 minutes at 20° C., the ATPase action was started by the addition of ATP. GSH in 10^{-3} M recovered 10% of the original activity and in 10^{-2} M 35% of that was restored. On the other hand, PCMB in a dilute concentration of $5–10 \times 10^{-6}$ M rather enhanced the ATPase activity by some 10–30% in the presence of 10 mM Ca as activator. The latter fact confirms the recent finding of Kielley and Bradley (1956) on rabbit myosin ATPase. These influences of PCMB and GSH show that SH groups may be involved in the active center of the ATPase action in the protein molecule.

Discussion

From the observations described above, the interaction of myosin B from crayfish tail muscle with ATP is quite the same as that of rabbit myosin B. The great extent of physical changes, e.g., super-precipitation, drop of viscosity or of turbidity with ATP, shows the main component of the present myosin B preparation was actomyosin. The salting-out analysis suggested that most of the proteins might be actomyosin and a minor part of them be myosin. There is no evidence to show the presence of tropomyosins as contaminants in the preparation. The thread prepared from crayfish myosin B contracted on addition of ATP. It is to be noted that myosin B from crayfish jaw muscle was very similar to that from tail muscle with respect to the ATPase activity, and super-precipitation and viscosity change with ATP.

Attention should be arrested to the pH optima of the ATPase activity of crayfish myosin B. The pH-activity curve in the presence of 10mM $CaCl_2$ and 0.6 M KCl is quite similar to that of molluscan myosin B (Maruyama, 1957a), not to that of insects (Maruyama, 1954). This is rather of the vertebrate type (*cf.* Engelhardt, 1946). In any case, two pH optima in the presence of Ca, activation by Ca and inhibition of the Ca effect by Mg in the ATPase action are all common to myosin B's from higher invertebrate and all vertebrate muscles so far reported (*cf.* Maruyama and Tonomura, 1957), although the former seems not to be the case in myosin ATPase from squid mantle muscle, where only the acid optimum was observed (de Villafranca, 1955).

Although essentially the same regards with the interaction between the contractile protein and ATP, one definite difference exists between actomyosins from invertebrate and vertebrate muscles. This is the absence of adenylate deaminase activity in invertebrate actomyosins. This fact was first discovered in insect muscle by Gilmour and Calaby (1953) and later confirmed in insects (Maruyama, 1957c), a sea-anemone (Maruyama, 1957d) and molluscs (Kitagawa and Tonomura, 1957; Maruyama, 1957a). These comparative studies may throw a strong doubt on the essential physiological role of adenylate deaminase in muscular activity. From the viewpoint of biochemical evolution, it is of much interest to investigate whether adenylate deaminase activity is present in prochordate muscles, especially in Acrania.

The relatively large quantity of easily available muscle of crayfish tail will make it a highly desirable material for a further study of the biochemistry and biophysics of the muscle contractile proteins from the comparative point of view. Crayfish myosin B was shown to possess the most similar peculiarities to rabbit in invertebrates so far known (*cf.* Maruyama and Tonomura). Especially the role of the guanidine kinase system in the recovery process of the ATP-induced physical change of the actomyosin solution, suggested by Tonomura's school (1956) in *Pecten* adductors, is expected to be elucidated more distinctly in crayfish tail muscle.

SUMMARY

1. Contractile protein (myosin B) was extracted and purified from tail muscle of the crayfish *Cambarus clarkii.*

2. Ultraviolet absorption spectra of crayfish myosin B dissolved in 0.6 *M* KCl showed a protein nature. The $\epsilon 275$ was 9 and $\epsilon 275/\epsilon 255$ was 1.3. Acid-soluble purine-pentose content was 1.5×10^{-5} mole per gram protein and nucleic acid purine-pentose content was 1×10^{-5} mole.

3. Crayfish myosin B was quite soluble in 0.4 *M* KCl. Salting-out analysis with ammonium sulfate indicated the main component is actomyosin.

4. Super-precipitation with ATP was clearly seen to take place in the range of 0.08–0.16 *M* KCl concentrations. The phenomenon was most distinguished in the presence of 0.1 *M* KCl and 10^{-5} *M* $MgCl_2$ at pH 7.0 at 25° C. EDTA, in concentrations higher than 10^{-4} *M*, completely inhibited the precipitation.

5. A drop of viscosity with ATP was observed in the presence of 0.6 *M* KCl. The ATP sensitivity of Weber and Portzehl was 100%. The recovery process was also observed to a considerable extent.

6. ATP also caused a drop of turbidity of the myosin B solution, the maximal drop reaching 50%.

7. The magnesium-activated apyrase activity and adenylate kinase activity were detected in the water-extract of crayfish muscle.

8. No adenylate deaminase activity was demonstrated in crayfish myosin B.

9. Crayfish myosin B had a true ATPase, activated by Ca. In the presence of 0.6 *M* KCl and 10 mM Ca at 30° C., the enzyme showed two pH optima, a higher one at 9.0 and a lower one at 6.0. In the presence of 0.6 *M* KCl at pH 7–8, EDTA maximally enhanced the ATPase action, Qp being higher than 3000. The activating effect of Ca was sensitive to the inhibiting action of Mg. The Michaelis constant was 5×10^{-5} *M* in the presence of 10 mM Ca and 1×10^{-3} *M* in the presence of 10 mM EDTA.

10. The ATPase action of crayfish myosin B was very sensitive to heavy metals, such as Cu or Zn. PCMB easily blocked the enzyme action. However, PCMB in dilute concentrations rather enhanced the Ca-activated ATPase activity.

LITERATURE CITED

BOWEN, W. J., AND T. D. KERWIN, 1954. A study of the effects of ethylenediaminetetraacetic acid on myosin adenosinetriphosphatase. *J. Biol. Chem.*, **291**: 237–247.

BUCHTHAL, F., A. DEUTSCH, G. G. KNAPPEIS AND A. MUNCH-PETERSEN, 1951. Further investigations on the phosphorus and nucleotide uptake of actomyosin. *Acta Physiol. Scand.*, **24**: 365–384.

COLOWICK, S. P., AND H. M. KALCKAR, 1943. The role of myokinase in transphosphorylations. 1. The enzymatic phosphorylation of hexoses by adenylpyrophosphate. *J. Biol. Chem.*, **148**: 117–126.

EDSALL, J. T., AND J. W. MEHL, 1940. The effect of denaturing agents on myosin. II. Viscosity and double refraction of flow. *J. Biol. Chem.*, **133**: 409–429.

ENGELHARDT, V. A., 1946. Adenosinetriphosphatase properties of myosin. *Adv. in Enzymol.*, **6**: 147–192.

Gilmour, D., and J. H. Calaby, 1953. Physical and enzymatic properties of actomyosins from the femoral and thoracic muscles of an insect. *Enzymologia*, **16**: 23–33.

Humphrey, G. F., 1948. The adenosinetriphosphatase activity of myosins from marine animals. *Physiol. Comp. et Oecol.*, **1**: 89–94.

Kielley, W. W., and L. B. Bradley, 1956. The relationship between sulfydryl groups and the activation of myosin ATPase. *J. Biol. Chem.*, **218**: 653–659.

Kitagawa, S., and Y. Tonomura, 1957. Contractile proteins from adductors of *Pecten*. III. Failure to detect adenylic acid deaminase activity. *J. Biochem. (Tokyo)*, **44**: 317–320.

Kominz, D. R., F. Saad and K. Laki, 1957. Vertebrate and invertebrate tropomyosins. *Nature*, **179**: 206–207.

Laki, K., 1957. Composition of contractile muscle proteins *J. Cell. Comp. Physiol.*, (in press).

Lohmann, K., 1935. Über die Aufspalung der Adenylpyrophosphorsäure und Argininphosphorsäure in Krebsmuskulatur. *Biochem. Zeitschr.*, **282**: 109–119.

Lohmann, K., and L. Jendrassik, 1926. Kolorimetrische Phosphorsäure-Bestimmungen im Muskelextrakt. *Biochem. Zeitschr.*, **178**: 419–426.

Maruyama, K., 1954. Studies on adenosinetriphosphatase from various insect muscles. *J. Fac. Sci. Tokyo Univ.*, IV, **7**: 231–270.

Maruyama, K., 1957a. Contractile proteins from adductors of *Meretrix* and *Cristaria*. *Annot. Zool. Jap.*, **30**: 63–66.

Maruyama, K., 1957b. Interaction of insect actomyosin with adenosine triphosphate. *J. Cell. Comp. Physiol.*, (in press).

Maruyama, K., 1957c. A further study of insect actomyosin. *Sci. Pap. Coll. Gen. Educ. Univ. Tokyo*, **7**: 213–241.

Maruyama, K., 1957d. Sea-anemone apyrase. I. Identification of the reaction products. *Enzymologia*, (in press).

Maruyama, K., and Y. Tonomura, 1957. Comparative studies of myosin B-ATP system. *J. Res. Inst. for Catalysis (Hokkaido Univ.)*, **5**: 55–70.

Mommaerts, W. F. H. M., 1948. The reaction between actomyosin and adenosine triphosphate. *J. Gen. Physiol.*, **31**: 361–375.

Moriwaki, K., 1956. Effects of azide on the ATP content of fish and toad embryos. M.S. Thesis. Univ. of Tokyo.

Sarkar, N. K., 1950. Observations on the glycerinated *Limulus* muscle. *Enzymologia*, **14**: 288.

Schneider, C., 1946. Phosphorous compounds in animal tissues. 3. A comparison of methods for the estimation of nucleic acids. *J. Biol. Chem.*, **164**: 747–751.

Snellman, O., and M. Tenow, 1954. A contractile element containing tropomyosin (actotropomyosin). *Biochim. et Biophys. Acta*, **13**: 199–207.

Szent-Györgyi, A., 1945. Studies on muscle. *Acta Physiol. Scand.*, **9**: Suppl. 25.

Szent-Györgyi, A., 1951. Chemistry of muscular contraction. Sec. Edition. Academic Press, Inc., New York.

Tarver, E., and M. F. Morales, 1951. The reaction between actomyosin and various nucleotides and phosphates, as followed by ultraviolet absorption. *J. Cell Comp. Physiol.*, **37**: 235–236.

Tonomura, Y., 1956. Physicochemical study on the actomyosin-adenosine triphosphate system. *J. Res. Inst. for Catalysis (Hokkaido Univ.)*, **4**: 87–108.

Tonomura, Y., H. Matsumiya and S. Kitagawa, 1957. The effect of ethylenediaminetetraacetic acid on the interaction between actomyosin and adenosine triphosphate. *Biochim. et Biophys. Acta*, **24**: 568–576.

Tonomura, Y., K. Yagi and H. Matsumiya, 1956. Contractile proteins from adductors of *Pecten*. II. Interaction with adenosine triphosphate. *Arch. Biochem. Biophys.*, **64**: 466–479.

Tsao, T. C., P. H. Tan and C. M. Peng, 1956. A comparative, physicochemical study of tropomyosins from different sources. *Scientia Sinica*, **5**: 91–111.

de Villafranca, G. W., 1955. Adenosinetriphosphatase activity of squid mantle muscle (*Loligo pealii*). *Biol. Bull.*, **108**: 113–119.

Weber, H. H., and H. Portzehl, 1952. Muscle contraction and fibrous proteins. *Adv. Protein Chem.*, **7**: 161–252.

Weber, H. H., and H. Portzehl, 1954. The transference of the muscle energy in the contraction cycle. *Progress in Biophysics*, **4**: 60–111.